Changes of Phosphorous compounds in Porcine Muscle Post Mortem

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Phosphorous compounds are very rich with energy and play an important role in the biochemical processes occuring in muscle post mortem. Adenosine triphosphate is of exceptional importance among them.

Changes in the contents of these compounds in animal muscles post mortem has been very often investigated during the last years /1,2,3,7/ However, the results of some of these investigations are very different. So, Fredholm /3/ has found that ATP decomposes very slowly in chilled pork meat, while Millo and al./7/ detected that all biochemical processes, including the changes in phosphorous compounds, were almost completed one hour after slaughter.

Taking into consideration that the function of the nucleotides in muscles post mortem is very important and that the results of investigations of changes in content of this compounds, as well as their participation in biochemical changes are different, require continuation of investigation in this sense.

Out of that reason we decided to examine what changes in quantity undergo of adenosine triphosphate /ATP/, adenosine diphosphate /ADT/, adenosine monophosphate and inosine monophosphate /AMP + IMP/, creatine phosphate /CP/ and inorganic phosphate /Pi/ in porcine muscle post mortem as well as the changes in pH and WHC.

Experiments

Material. Lumbal parts of longissimus dorsi of white fleshy pigs were used. The samples were taken from carcasses of the pigs live weight of loo to 120 kgs., aged from 6 to 8 months.

The pigs were stunned by electric current, then bleeded and dressed in ordinary way.

In I experiment /five samples/ the contents of ATP, ADP, and AMP + IMP, as well as pH and WHC were measured several times during 33 hrs! post mortem.

In II experiment /five samples/ ATP, ADP, AMP +IMP, pH, WHC as well Pi and CP were also measured several times during 24 hrs post mortem. Besides it, the myoglobin content has been also determined.

The samples were kept at $+4^{\circ}C$ during examination.

Methods. ATP,ADP and AMP+IMP were separated on a colone /1x2 cm/ with anion exchange resin Dowex 1x8, by the method of Cohn and Carter /6/. These compounds were eluated with 150 ml of 0,2 M NaCl and 0,1 N HCl, 0,02 M NaCl and 0,1 N HCl, and with 0,03 N HCl, respectively. The concentration has been determined by measuring the extinction of eluate at 257 mμ /8/.

P_i and P_i + CP were determined by the method of Berenblum and Chain, modified by Ennor and Stocker /6/. Phosphate were extracted by isobutanole, and the colour developed by molybdate reagent. The concentration was determined by measuring the extinction of extract at 610 mμ and calculated at the phosphate content /as ortho phosphate/. The extinction in both cases was measured by spectrophotometer "Beckman" DU, modell G-2400.

Myoglobin was determined by method of Hart /5/ in aqueous extract and the content was expressed as extinction at 540 mu.

WHC was determined by method of Grau and Hamm /4/, and the pH measured in aqueous extract /1:10/ with glass electrod of Phillips' pH-metre, modell 9400.

Results and Discussion

From the results of I experiment /Fig. 1/ one can see that the average value for the content of ATP after 1,5 hr was 2,53 uM/g of fresh muscle tissue and that it was rapidly lowered to 1,20 uM/g during 6 hrs post mortem. After that time ATP lowered slowlier up to 24 hrs post mortem, and then almost insignificantly up to 33 hrs.

In II experiment /Table 1, and Fig. 2./ it was detected that the average value of the content of ATP during the 1. hr post mortem was 3,32 uM/g, but rapidly lowered at 0,99 uM/g up to 8 hrs post mortem and kept decreasing up to 24 hrs.

By extrapolation in order to get the approximate initial ATP concentration one gets the value of 3,1 uM/g in I experiment, and 3,8 uM/g in II experiment.

In relation to the initial extrapoleted values ATP was decomposed during first 9 hrs for about 60 to 75% and during 24 hrs post mortem for about 80 to 95%.

The significant increase of ADP was detected in I experiment /Fig. 1/ up to 4 hrs post mortem /from 1,94 to 2,29 uM/g/ and then was registered almost at the same level up to 24 hrs post mortem. Content of AMP + IMP was significantly increasing until 24 hrs./from 0,60 to 1,40 uM/g/ and then again lowered. After that time ADP was remarkably lowering, while AMP + IMP, in the contrary, lowered very slightly. In II experiment /Table 1. and Fig. 2./ the significant increase of ADP was detected during 24 hrs. /from 2,19 60 3,71 uM/g/ while the content of AMP+IMP remained almost at the same level.

The content of inorganic phosphate was increasing significantly during 8 hrs. post mortem/from 0,71 to 0,82 mg/g/and then slowlier until the end of examination. /Table 1./ The difference between P_i and P_i+CP shows that the level of CP was lowered, but could be detectable after 24 hrs. post mortem.

Average initial pH_{90} value in I experiment was 6,04 and in II experiment /pH_{60}/ 5,98 which is an indication that the examined muscles may be considered as that of normal quality. This presumption support also average values of WHC detected in both experiments - in I one WHC was 5,7 cm^2 after 1,5 hr post mortem, and in II 5,02 cm^2 after 1 hr post mortem. WHC after 24 hrs post mortem was 7,31 and 9,66 cm^2, respectively.

One can say that initial concentration and the rate of decomposition of ATP were fairly similar in both experiment. However, the changes in content of ADP and AMP + IMP differ among themselves - in I experiment the content of ADP was significantly changed during investigation, while the content of AMP + IMP significantly increased in some relation to decomposition of ATP; in II experiment the conditions were opposite, because the content of AMP + IMP was insignificantly changed while the content of ADP increased in some relation to the decomposition of ATP.

These differences may be probably the result from the different characteristics of the inidvidual samples in I and II experiment. Namely, in I experiment there were the samples with the pH_i below 6,0, then with the significant differencesin initial concentration of ATP, and in II experiment there was one of the five samples with pH_i 5,55 WHC 8,25 cm^2 and the content of the myoglobin was low /E_{540}= 0,095/. Probably, by investigation a greater number of samples, classified in groups according to, for example, the rate of the pH changes if could be possible to explain the causes of these differences.

However, the both experiments had in common rapide decomposition of ATP which occured from 6 to 8 hrs. post mortem, and after 4 hrs. reached somewhere about 50% of initial concentration. After 24 hrs. post mortem was found less than 20% of undecomposed ATP in both experiments.

If we compare the obtained results with the corresponding ones which can be found in literature, we can see that in our experiment the initial concentration of ATP was very similar to that found by Millo and al. /7/, and significantly lower than that detected by Bendall and al. /1/ and Bodwell and al. /2/. -5-

The rate of decomposition of ATP in our experiment neither corresponds with the results obtained by Fredholm nor with those found by Millo and al. because, according to Fredholm /3/ ATP has not at all been decomposed during first 24 hrs. post mortem, and according to Millo and al. /7/ it was completely decomposed in a couple of hours post mortem. These authors found similar initial concentration of ADP as we did, but according to them this concentration significantly lowered below the value we found after several hours post mortem. On the contrary, detected amount of AMP was fairly similar to the amount of AMP + IMP we found, except for a couple of hours immediately post mortem, when Millo and al. /7/ registred much higher values.

According to the differences between P_i and P_iCP it can be concluded that, in our experiment, the content of CP was decreasing slowlier then it was detected by Bendall and al. /1/ and by Bodwell and al. /2/ who didn't even detect the CP in some of the samples immediately after the death of the animal.

S U M M A R Y

In this work, in two experiments, which consisted of five samples each, there have been examined the changes in contents of ATP, ADP, AMP + IMP, pH and WHC during 33 hrs. post mortem /I experiment/, and ATP, ADP, AMP+IMP, P_i, CP, pH and WHC during 24 hrs post mortem / II experiment/, respectively.

By extrapolation the obtained average results it was determined the initial content of ATP, and for the I experiment it was 3,1 uM/g of fresh muscle tissue and for the II experiment it was 3,8 uM/g. Initial content of ATP as well the rate of decomposition of this compound were fairly similar in both experiments /for I experiment Fig. 1. and for II experiment Table 1. and Fig. 2./. During first 4 hrs. post mortem the content of ATP reached about 50% of ATP of initial value, and after 24 hrs it lowered up to less than 20% of this.

The rate of changes in content of ADP and AMP+IMP was different in both experiments.

CP was detected in all examined samples /II exp./ and was also found 24 hrs post mortem.

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RÉSUMÉ

Dans ce travail, on a en deux éxpériences de cinq échantillons, examiné les changements en ATP, ADP, AMP + IMP, pH et le pouvoir de rétention d'eau au cours de 33 heures / $I^{ère}$ expérience / et en ATP, ADP, AMP + IMP, P_i P_i + CP, pH et le pouvoir de rétention d'eau, au cours de 24 heurs / $II^{ème}$ expérience/.

On a déterminé la teneur initiale en ATP par extrapolation des résultats obtenus, et elle était en moyenne chez les échantillons de $I^{ère}$ expérience 3,1 uM/g sur muscle frais, et chez les échantillons de la $II^{ème}$ expérience 3,8 uM/g.

Des résultats obtenus on voit que la teneur initiale, ainsi que le cours de la décomposition de l'ATP sont assez semblables dans les deux expériences /$I^{ère}$ exp. fig. 1. et $II^{ème}$ tab. 1 et fig. 2./.

La teneur en ATP atteint au bout de la $4^{ème}$ heure post mortem environ 50% de valeur initiale, et après 24 heures est décomposée plus que 20% de ce nucléotide.

Le cours des changements en teneur en ADP et AMP + IMP est différent dans ces deux expériences.

CP est trouvé dans tous les échantillons examinés /$II^{ème}$ exp./, et déterminé même 24 heurs post mortem.

REFERENCES

1. Bendall, J.R., O. Hallund and J. Wismer-Pedersen, 1963: Post mortem changes in the muscles of Landrace pigs, J.Food Sci., 28, 156.

2. Bodwel, C.E. et al., 1966; Post mortem changes in muscle, II Chemical and physical changes in pork, J.Food Sci., 31, 1.

3. Fredholm, H., 1963: 5 Mono, Di and Triphosphates of adenosine in beef and pork kept under refrigeration, IX Conf. of Europ. Meat Res. Workers, Budapest.

4. Hamm, R., 1960: Biochemistry of meat hydration, Adv. in Food Res., 10, 355.

5. Hart, P.C., 1961: De Bepaling van de Vleeskleur door Meting van de Extinctiewaarde, Tijdschrift voor Diergeneeskunde, 5.

6. Hawk, P.B., B.L. Oser and W.H. Summerson, 1954: Practical Physiological Chemistry, McGraw-Hill Book Comp. Inc., London.

7. Millo, A.E. Schilling und J. Bader, 1964: Über postmortale Prozesse in Muskel, Die Fleischwirtschaft, 6, 507.

8. Morell, S.A. and R.M.Bock, 1954. Am. Chem. Soc. 126th Meeting, New York.

Means of the quantities of ATP, ADP, AMP+IMP, P_i P_i+ CP, pH and WHC in pigs long. dorsi muscle /II experiment/

Time after death	ATP+	ADP+	AMP+ + IMP	P_i ++	P_i + PC ++	pH	WHC +++
1	3,32	2,19	0,71	0,71	0,76	5,98	5,02
4	1,89	2,84	0,75	0,77	0,81	5,75	7,61
8	0,99	3,19	0,85	0,82	0,84	5,66	8,95
24	0,45	3,71	0,80	0,89	0,90	5,56	9,66

\+ in u mols/g of fresh muscle tissue.

++ in mgP/g of " "

+++ in cm^2 of wet area

Table 1.

Fig. 1. Changes of ATP, ADP, AMP + IMP, pH and W.H.C. in long. dorsi muscle

(I experiment)

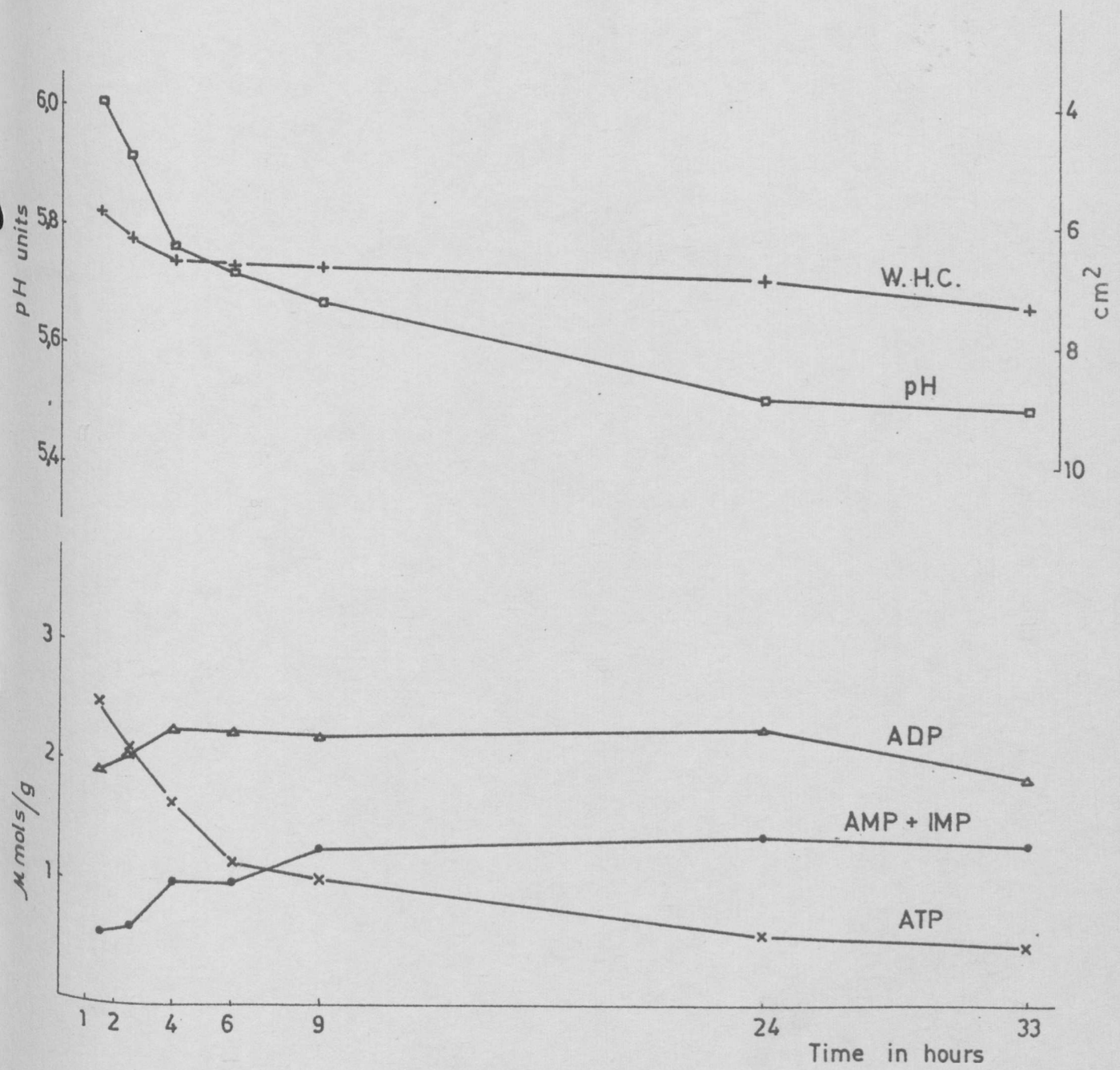

Fig. 2. Changes of ATP, ADP, AMP+IMP, P_i, P_i+ PC, pH and W.H.C. in long. dorsi muscle

(II exsperiment)